Perspectives in Science (2015) 6, 15—26

ScienceDirect

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The logic of metabolism☆

Antoine Danchin*, **Agnieszka Sekowska**

AMAbiotics SAS, Brain and Spine Institute, Hôpital de la Pitié-Salpêtrière, 75013 Paris, France

Received 17 September 2014; accepted 2 May 2015
Available online 23 October 2015

KEYWORDS
Metabolism;
Synthetic biology;
Paralogous metabolism;
Compound asymmetry;
Chemical reaction;
Cell dynamics

Summary Metabolism is often perceived as a fairly haphazard intertwined collection of chemical compounds that needs to be memorised by heart. The apparent absence of logic in its organisation dissuades most investigators to enter its arcanes. Yet, metabolism, that results from highly selective constraints is logically organised. The atoms of life must build up stable covalent bonds. Symmetry breaking is the rule. Proteinogenic amino acids are of the L-enantiomer type, and this will imply that carbohydrates are of the D-type. Water is ubiquitous because it favours entropy-driven shaping of macromolecules. Then, being the bathing medium of life, hydrolysis is the driving force in orientating pathways. Phosphates, in water, are metastable towards hydrolysis, and this makes them the ultimate energy quantum and currency. Making a variety of reactive molecules in the same retort implies either compartmentalisation or a protection/deprotection procedure, as in the laboratory of all chemists.

Contents

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* Corresponding author at: AMAbiotics SAS, Brain and Spine Institute, Hôpital de la Pitié-Salpêtrière, 75013 Paris, France. Tel.: +33 1 5727 4429; fax: +33 1 5727 4586.
E-mail address: antoine.danchin@normalesup.org (A. Danchin).

http://dx.doi.org/10.1016/j.pisc.2015.05.003

Introduction: a top-down view of synthetic biology

Within the realm of natural sciences biology is all about trapping and taming what we use to name ''information''. Remarkably, while the word is commonplace in biological studies it is not taken as seriously as in physics, where information is recognised as an authentic currency of Reality (Vedral, 2010). Yet, as seen in the image of Theseus' ship (which remained apt to float while its boards were replaced as they rotted away), the very existence of metabolism — where matter is in constant flux while the living entity carries on — tells us that information is at the core of life. A way to have access to its role is to reverse the research programme that created molecular biology (i.e., decompose living organisms into basic building blocks and try to reconstruct the organism bottom—up) following instead the top-down reasoning of the engineer when he or she aims at designing a new device. What should we take great care not to miss if we had to construct a machine endowed with life? This entails proceeding from top to bottom, with emphasis on dynamic processes and interactions between parts (Danchin and Sekowska, 2013).

We apply here this view to metabolism, the core dynamic process that unfolds life's attributes, made of the chemical transformations that keep going on as life proceeds. However, exploring the logic of metabolism would certainly require the writing of a very large book. This cannot be the purpose of this article. We have therefore limited the scope of our views, depicting only some of the logical constraints that give the general flavour of the rules that drive metabolism. We hope that this will allow the reader to see metabolism as a logically organised set of chemical reactions driving the processes which make life as we know it.

In this limited set we retained:

- basic constraints: a choice of atoms (allowing the making of stable aperiodic polymers in water)
- asymmetry, as the root of information: stereochemistry (e.g., L-aminoacids, D-sugars)
- anabolism (going forward via hydrolysis)
- after the feast: coping with leftovers

and finally, because the passing of time spoils all things:

- coping with inevitable errors (paralogous metabolism)

Basic chemical constraints

The atoms of life

The elements we find in living organisms are but a fraction of those in Mendeleieff's Table (Fig. 1). This is not by chance. Life develops in water at a temperature of approximately 300 K. A core property of its ingredients is that besides a limited number of small molecules (a few tens of atoms), life is made of macromolecules, giant polymers obtained by removal of a water molecule between residues chosen among a small alphabet of basic building blocks, twenty amino acids and five nucleotides. This simple arrangement allows for the necessary information management which is at the root of life: while the backbone of these polymers (a sequence of peptide or phosphodiester bonds) is invariable, the side chains may be arranged in an infinite variety of combinations. Still, making macromolecules implies considerable physico-chemical constraints because this requires the repeated formation of stable covalent bonds. In a covalent bond electrons share their presence between two or more atoms' nuclei. The electrons associated to a nucleus follow the rules of quantum physics laws, with energy levels that can be arranged along rows and columns, according to the way they match with the charge of their cognate nuclei.

As the rank of the row increases, the (negative) electrons of the outer shell of the atom become more and more loosely tied to the (positive) nucleus. When the outer shell is complete the atom is unreactive (this corresponds to the so-called rare gases). In a nutshell, this arrangement makes that the best candidates for building stable covalent bonds are, besides hydrogen, some of the atoms present in the second row of the periodic table. Further down, the atoms involved are mainly involved in electrostatic bonds (much weaker than covalent bonds) and in electron exchanges (redox reactions). Another constraint, somewhat anecdotal and often ignored, involves the logic of history. It concerns three atoms: lithium, beryllium and boron. All three are rare in the universe for cosmological reasons: they were generated in a limited amount by nucleosynthesis during the first stages of our universe's formation (Bernas et al., 1967). Finally, fluorine, if present in biomolecules, is exceedingly rare (Ayoup et al., 2014). This is because carbon—fluorine bonds are extremely stable and cannot be metabolised easily. As a consequence, the primary atoms of life are restricted to hydrogen, carbon, nitrogen and oxygen (typically the atoms combined in

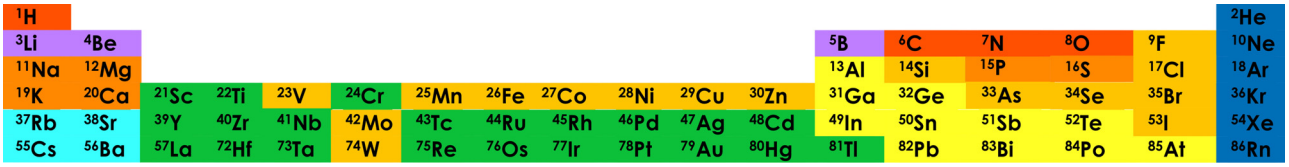

Figure 1 The periodic table of elements, with emphasis on life-related atoms. All elements found in life are shown in orange. The four central elements of life, hydrogen, carbon, nitrogen and oxygen are shown in deep orange. Elements that are cosmologically rare are in purple. Rare gases are in deep blue. Metals involved in central electrostatic interactions are in medium orange, together with chlorine, that is used as a counter-anion in many fluids outside cells. Some elements (e.g., arsenic, fluorine, bromine, iodine, silicium or tungsten) are used only in a limited number of species.

extra-terrestrial molecules, when they are found). An interesting endeavour in synthetic biology would be to try and include boron in the picture.

As shown in the figure, other atoms are also involved in living organisms. Most, in fact, play important roles in specific features of catalytic reactions needed to construct, modify and destroy covalent bonds, electrostatic interactions (metals) and more or less complicated electron exchanges (transition metals and complex anions such as molybdate or tungstate). Arsenic can be found in some rare arsenolipids or arsenosugars, for example (Couture et al., 2012). Two outliers have to be accounted for because they are ubiquitously present in living cells: sulphur (the higher homolog of oxygen) and phosphorus (the higher homolog of nitrogen). The former, being in a lower row than oxygen, is mainly involved in electrons trafficking (its biologically-relevant redox potential varies from −2 to +6), in formation of energy-rich thioesters and via a remarkable affinity for iron, in making iron-sulphur clusters that probably had a seminal role on the origin of life on earth, where it is quite abundant (Wickramasinghe, 1973). The latter, phosphorus, is the candidate that Wolfe-Simon and colleagues proposed to see replaced by arsenic in an incredibly ludicrous article that delighted the ignorant pursuit of mass media (Wolfe-Simon et al., 2011). Phosphorus has been retained as a preferred element for life because of a remarkable property of phosphates (not shared by arsenates): polyphosphates can be hydrolysed, but this requires a very high activation energy, allowing phosphates to behave as the basic energy currency, able both to be stored and exchanged (Westheimer, 1987).

''Dissymmetry, this is life'' (Louis Pasteur)

With these atoms as building blocks a huge number of small molecules can be constructed. However, life has retained a small number of those, building its informational requirements on the construction of macromolecules with a common skeleton, made of peptide bonds for proteins and on phosphodiester bonds for nucleic acids. Everyone is aware that a great many compounds that make the cell's metabolism are asymmetric. With their alpha-carbon atom linked to an amino- and a carboxyl-group amino acids can be split in two major enantiomers, L- and D-. Proteins are composed of L-aminoacids. Whether this is the result of a purely contingent accident of prebiotic life or not has been the subject of much discussion, for fairly irrational reasons. Indeed, symmetry breaking is a common property of physical systems — it is where information steps in — and there is certainly no need to try and look for a compelling cause for the presence of L-amino acids in extant proteins. Symmetry had to be broken in the amino acid choice for building polymers, exactly as we have to drive on the right or on the left in order to prevent collisions or impossible traffic jams. Once a side has been chosen, only a dictator could reverse it, as Napoleon did, changing road riding from left to right.

We can thus take for granted that proteinogenic amino acids are of the L-type and accept that this is contingent. By contrast, carbohydrates in cells are most often based on a D-stereoisomer skeleton. This is no longer a random pick. Carbohydrate asymmetry is driven by the asymmetry of amino acids. Indeed, there is a strong selective advantage in avoiding chemical interference with the costliest process of life, translation of messenger RNAs into proteins (Russell and Cook, 1995). Glycerate and its variants are core metabolites of central carbohydrate metabolism. L-glycerate closely mimicks L-serine and, were it present at a substantial concentration, it would slow down many of the processes where this amino acid is involved. It is easy to understand that this created a strong pressure for favouring its enantiomer D-glycerate. Indeed, L-glycerate is generally toxic in extant living systems (Zhu and Lin, 1987). As a consequence, the central carbon pathways giving rise to gluconeogenesis, the pentose pathway and related pathways naturally led to the ubiquitous D-carbohydrates.

The role of specific stereoisomers is central in life, and specific enzymes can choose between one or the other. For example, methionine sulphoxide reductase A uses the *S*-methionine sulphoxide epimer, while methionine sulphoxide reductase B uses the *R*-epimer (Lee and Gladyshev, 2011). Remarkably, the two reductases have catalytic sites that more or less mirror each other, displaying a fairly extraordinary scenario of mirror-symmetry convergent evolution (Lowther et al., 2002). While it is essential in driving metabolism, this constraint in the management of symmetry is often overlooked. Yet, asymmetry may be involved in crucial structures that have a considerable impact on evolution. Based on asymmetry, an interesting instance of the Red Queen process that drives a good deal of evolution may have been opened up in the early times of life. We know that membranes differ between Bacteria and Eukarya on the one hand and Archaea on the other hand. The former have a skeleton of phospholipids based on *sn*-glycerol-3-phosphate (Yao and Rock, 2013; Wendel et al., 2009). The latter have a very similar lipid bilayer, but it is based on ethers (Koga et al., 1993), rather than esters, and, remarkably, on *sn*-glycerol-1-phosphate (Nishihara et al., 1999), which — and

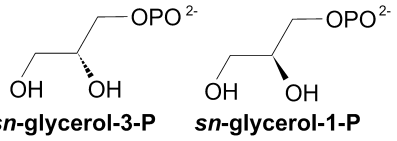

Figure 2 Two enantiomers of glycerol-phosphate make the backbone of the lipid bilayer in biological membranes. Bacteria and Eukarya use *sn*-glycerol-3-phosphate while Archaea use *sn*-glycerol-1-phosphate.

this is not apparent in the standard nomenclature — is the direct enantiomer of *sn*-glycerol-3-phosphate (Fig. 2).

This chemical discrepancy is consistent with a chemically driven scenario of the origin of life, where the first cells were phagocytes which explored their environment by engulfing other cells, either creating compartmentalised structures or degrading them as food sources. This widespread situation opened up an escape way: cells could either make strong and robust envelopes to escape predation (and this led to the Bacteria), or they had membranes that could not fuse with those of their putative predators, allowing them to break loose, and this led to the Archaea (Danchin, 2014). This possibly explains why cells which belong to the Archaea domain are never pathogens. Yet they are present everywhere, and some animals are reservoirs of many of their species (e.g., termites or bovines, with their complex gut).

Current metabolites: why the twenty proteinogenic amino acids?

Twenty amino acids (twenty-two with selenocysteine and pyrrolysine) make the skeleton of all proteins. Several, such as glycine, alanine, serine or aspartate are quite simple and indeed easily formed under fairly mild chemical conditions. But others, such as the aromatics phenylalanine, tyrosine and tryptophan, or the basic amino acids histidine, lysine and arginine are much more complicated and would require fairly intricate hypotheses to explain how they could be generated spontaneously. This small set of amino acids has been selected for because of the specific role of their side chains, charged, or hydrophobic, but also displaying specific interaction qualities such as in aromatics (Pascal et al., 2005).

As another case in point, methionine, with its long straight chain, behaves as a drop of oil. Yet, this role could have been performed by norleucine, and indeed norleucine is not very toxic and can replace most methionines in proteins when made available to cells (Barker and Bruton, 1979). Why was methionine retained, then? The short answer highlights a general constraint that contributes to explain why proteins are made of the extant amino acids: history. Indeed, methionine is likely to have existed very early on, when sulphur was ubiquitous in prebiotic syntheses (Danchin, 1989; de Duve, 1992; Wachtershauser, 2007). It became a core constituent of the ubiquitous metabolite, *S*-adenosyl-methionine (AdoMet), which is the universal methyl-group donor, as well as the precursor of the amino propyl-groups important for polyamine biosynthesis (Sekowska et al., 2000). Norleucine could not replace methionine as it would have limited its role to providing the ''lubricant'' function of methionine in proteins. This precluded it to substitute for methionine in its general metabolic functions.

Several other L-amino acids are present in the core cell metabolism. Why are they not found in proteins? Here, a logic of another type substitutes for the constraints of history: with many non-proteinogenic amino acid chemistry imposes considerable limitations to the reactions that may unfold in particular in the translation process. For instance, homoserine, homocysteine and ornithine are rejected from proteins. Yet they are common components of non-ribosomal peptide synthesis (Cheung et al., 2009). Their rejection from proteins is accounted for by the existence of an inevitable obstacle when they are activated during translation: they cyclise and abort the process (Jakubowski and Fersht, 1981). Other omissions, such as the lack of L-alpha-aminobutyrate in proteins are more difficult to explain. This amino acid is indeed a common by-product of branched-chain amino acids biosynthesis (Hofgen et al., 1995) and the reason for its absence from proteins is not immediately apparent. It may be that its similarity with L-alanine does not provide it with sufficient advantage to have recruited specific tRNAs and codons, certainly a very costly process in terms of the number of genes involved.

Chemistry and time: going forward

A central rule of life is that it keeps going. While the vast majority of biochemical reactions are close to equilibrium and can go in either direction depending on the availability of the substrates/products, some impose the direction of the overall metabolism. The dominant agent here is water because, in molecular terms, it is in very large excess (of the order of 50 M) as compared to any of the other chemicals that make the cell's metabolism. This implies that hydrolysis displays a considerable kinetic power, driving reactions forward. We illustrate this fact here with two examples, hydrolysis of pyrophosphate, and deamidation of 2-ketoglutaramate.

Pyrophosphate hydrolysis and phosphorolysis

Pyrophosphate hydrolysis is a well-known process but, despite the fact that pyrophosphatase is an essential enzyme, its impact is underestimated. Indeed, many metabolic reactions (more than 500 are listed in the KEGG database, Tanabe and Kanehisa, 2012) produce pyrophosphate (PP_i). This is illustrated by synthesis of a wealth of small molecules (e.g., building blocks such as asparagine, sulfate assimilation enzymes, regulators such as cyclic AMP, etc.) or of macromolecules (e.g., synthesis of nucleic acids produces pyrophosphate, and, in fact, an excess of pyrophosphate will lead polymerases to go backward instead of forward, Liu and Sommer, 2004). Hydrolysis of pyrophosphate is probably the major driver of the cell metabolism. Remarkably, despite its apparent outcome in wasting energy, this reaction is not always linked to means to recover the energy stored in the molecule as would be the case if pyrophosphate hydrolysis were coupled to some vectorial transport. This suggests that involvement of this product of so many reactions is decisive as a general agent driving metabolism forward. So much so that it was difficult even

during the long time of evolution to reconcile energy production and keeping things in control (in general life prefers to lose energy rather than to lose control). Nevertheless, in several organisms, some specialised structures associate a membrane pyrophosphatase and vectorial transport, and this is presumably an ancient feature of metabolism (Tsai et al., 2014).

With pyrophosphate in mind we may observe that there is a chemical logic in the distribution of the various roles of mono-, di- and triphospho-nucleosides in metabolic pathways. The energy loaded in the phosphate bond between the alpha-, beta- and gamma-positions is not available in the same way. Pyrophosphate production results from hydrolysis of NTPs between their alpha- and beta-phosphate groups. The alpha-bond is fairly stable and cannot be readily used as an energy currency (an exception is found in the two steps synthesis of AdoMet from methionine and ATP, presumably a very ancient reaction (Pajares and Markham, 2011), where both phosphate and pyrophosphate are produced, the overall reaction being driven by pyrophosphate hydrolysis). By contrast, the beta- and gamma-phosphates can be readily mobilised, releasing a similar amount of energy. Why, then, three phosphates in nucleotides, not just two? The energy of the beta-phosphate of ADP or GDP can be used in phosphorylation, as shown in some reactions (Castro-Fernandez et al., 2014). Yet, this is the exception rather than the rule. Indeed, the need to proceed forward requires a kinetically irreversible step. This step is provided by pyrophosphate hydrolysis, as just discussed. This implies that a pyrophosphate molecule is produced when using the energy carried by nucleotides. Because of the energy availability difference between the first alpha-phosphate group and the subsequent groups, this requires that the nucleotides have no less than three phosphates. In fact, while nucleotides are commonly used as NTP → NDP + activated phosphate, many reactions use NTPs in the form NTP → activated NMP + PP_i, with activated NMP as an intermediate in a further reaction, such as in the transcription and replication processes, or in synthesis of dTMP (irreversibly coupled to elimination of dUTP in order to prevent U from getting into DNA, Fig. 3). Interestingly, identifying reactions of this type might be used as the signature of reactions present in ancient metabolism. The corresponding exploration has not yet been undertaken.

This chemical logic opens a checkpoint at the level of phosphate availability. This permitted evolution to develop a remarkable process, phosphorolysis, that allows recovery of energy-rich phosphate bonds while breaking down molecules. Acting as a substitute for hydrolysis, phosphorolysis is central in the metabolism of nucleotides. Polynucleotide phosphorylase, a ubiquitous enzyme present in the bacterial degradosome and in the exosome of Archaea and Eukarya, uses phosphate to break down nucleic acids into NDPs, the energy-rich counterparts of the NMPs produced by hydrolysis (Lin-Chao et al., 2007). Now, NDPs are the ubiquitous precursors of deoxyribonucleotides, and this offers an explanation for why they are almost exclusively made from ribonucleoside *di*phosphates not *tri*phosphates (Ahmad and Dealwis, 2013). Using nucleoside diphosphates instead of triphosphates also fits with their lower supply in the cell, which is a better match with the amount of DNA as compared to that of RNA. Finally, production of nucleoside diphosphates solves an intriguing riddle of pyrimidine

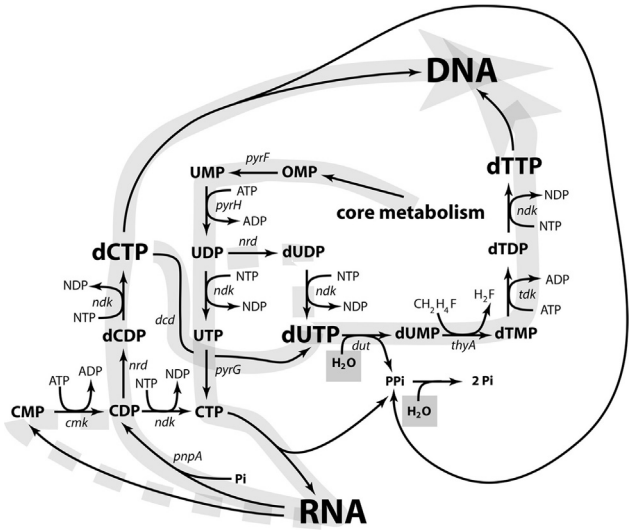

Figure 3 Excerpt of pyrimidine nucleotide metabolism pathways. Hydrolysis of pyrophosphate is the driving force of nucleic acids synthesis. It is also essential for the formation of thymidylate. Synthesis of deoxycytidylate requires RNA turnover and phosphorolysis, managed by polynucleotide phosphorylase (PnpA) is used to spare energy.

biosynthesis (Fig. 3), because it drives synthesis of dCDP (subsequently dCTP) under conditions when the de novo synthesis of CTP does not proceed through a CDP step—required as a substrate of ribonucleotide reductase — but comes directly from transamination of UTP (Nitschke et al., 1998). This intriguing metabolic feature also reveals the presence of an unexpected coupling between RNA turnover and DNA synthesis, an observation that should be taken into account in all system biology studies modelling metabolism.

Deamidation as a forward reaction

Chemical reactions in living cells are essentially reversible. Some are driven to a particular state when a substrate is in great excess relative to the local availability of other metabolites. Such is the case of reactions involving water as a substrate. Reversibility is taken into account in the logical organisation of metabolism when the cell decides between constructing biomass or catabolising available nutrients. A molecule does not have a label attached to it telling the cell whether it will be used in an anabolic process or be degraded to provide particular groups or atoms. Amino acids are cases in point: they may be chained into polypeptides during protein synthesis or used as carbon, nitrogen (or sulphur) sources. The core reaction in the process is generally a transamination step, at the expense of ubiquitous donors/acceptors generated by the TCA cycle, aspartate/oxaloacetate and glutamate/ketoglutarate. In this process an alpha-keto-amino acid precursor (for example alpha-keto-isovalerate) will be modified into an amino acid (in this case L-valine) by a relevant aminotransferase. Amino acids may also be used as carbon and nitrogen sources, and in this case the aminotransferase reaction will yield an alpha-ketoacid, which, often, will subsequently be decarboxylated, while the decarboxylated remaining

of the molecule will be carried over via formation of an acyl-CoA derivative (in the case of valine, isobutyryl-CoA). The overall metabolic fluxes will be determined by the local relative amounts of the various molecules involved. Triggering biomass construction will favour production of most amino acid, while those used as carbon or nitrogen sources will be fuelled into catabolic processes. Yet, in some cases, it might be awkward or deleterious for the cell to degrade an amino acid that is a costly resource. Making transamination of methionine irreversible in the direction of methionine synthesis (to prevent loss of this costly sulphur-containing metabolite) illustrates another feature of the logic of metabolism.

While sulphur is ubiquitous in biomolecules, it is a highly reactive atom, very sensitive to the redox potential of its local environment. As a consequence, sparing processes that enable the cell to keep a sulphur atom in its final chemical and electronic environment (inside well characterised groups of atoms) often exists in cells (Sekowska et al., 2000). Methionine is a central metabolite of all living organisms. It is the precursor of *S*-adenosylmethionine (AdoMet). It is used in initiation of protein synthesis and it is a major residue in proteins, with specific functions such as copper binding (Maryon et al., 2013; Long et al., 2012). Its synthesis is costly (either via the inefficient, hence abundant, methionine synthase MetE (Li et al., 2014) or using MetH, a B12-dependent enzyme, very costly in genetic terms (Randaccio et al., 2010)), and its precursor homocysteine is toxic. The fate of AdoMet, which can donate each one of its sulphur-linked groups: methyl-, aminobutyryl- (usually, after decarboxylation as aminopropyl-), and adenosyl- (ribosyl-) illustrates how methionine is spared via a methionine salvage pathway. Methyl-transfer results in the synthesis of *S*-adenosyl homocysteine (AdoHcy), which is recycled or further metabolised, either after excision of the adenine moiety by hydrolysis catalysed by methylthioadenosine/*S*-adenosylhomocysteine nucleosidase (Parveen and Cornell, 2011), or by hydrolysis into adenosine and homocysteine by *S*-adenosylhomocysteine hydrolase (Turner et al., 2000) (Fig. 4). When the aminopropyl-group is transferred during synthesis of spermidine and spermine after decarboxylation of AdoMet, methylthioadenosine (MTA) is formed. This same by-product is also produced by *N*-acylhomoserine lactone synthases for quorum sensing in Gram-negative bacteria (Schuster et al., 2013) and 1-aminocyclopropane-1-carboxylate (ACC) synthase in plants (and possibly in bacteria producing ethylene, Lin et al., 2009). Furthermore, other still unknown reactions might exist, transferring the aminopropyl-group to yet unrecognised acceptors (most likely to be nitrogen atoms).

Recycling the sulphur-containing compounds *S*-adenosylhomocysteine and MTA is a challenge for all cells. Remarkably, MTA metabolism regenerates methionine in the process (Sekowska et al., 2004). While salvage proceeds first via either hydrolysis or phosphorolysis of MTA, recent work has established that there is considerable variation in the first steps of the pathway, with sometimes

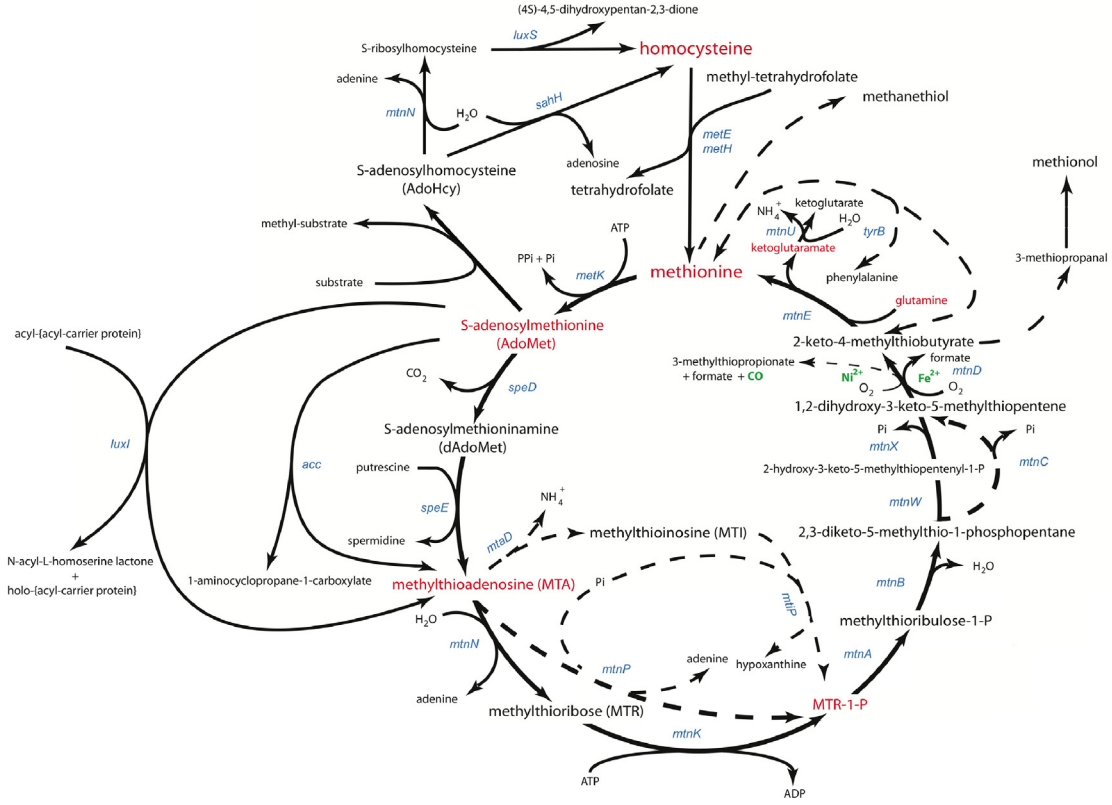

Figure 4 Summary of the methionine salvage pathway. While methionine and aromatics may be equilibrated via TyrB aminotransferase, MtnE aminotransferase uses glutamine as the alpha-amino donor, producing ketoglutaramate which is hydrolysed into ketoglutarate in an irreversible step.

deamination of MTA followed by phosphorolysis of methylthioinosine (Guan et al., 2012). The downstream steps are also variable, but a common final product is the immediate precursor of methionine, 2-keto-4-methylthiobutyrate (KMTB) (Fig. 4). This keto acid is readily transaminated into methionine. However, in the cases where the reaction has been explored, it appeared that *glutamine* was used as the alpha-amino group donor, not *glutamate* (Berger et al., 2003). This is very surprising because the reaction would produce alpha-ketoglutaramate, a molecule that is not part of standard metabolic pathways and possibly toxic (Cooper and Kuhara, 2014). Analysis of the genes co-evolving with the pathway showed us that an omega amidase (MtnU) is usually present in parallel with the methionine salvage pathway. This enzyme hydrolyses alpha-ketoglutaramate in to alpha-ketoglutarate and ammonium, solving the riddle of the requirement for glutamine. The logic of this step comes to the fore: because this is a hydrolytic reaction, it drives the methionine salvage pathway forward, precluding degradation of methionine (Belda et al., 2013).

An example of functional logic: coping with leftovers

The logic of metabolism is also constrained by essential functions which can be revealed using functional analysis (Fantoni et al., 2009). For instance, management of waste is of major importance. Among the many related processes, we find trashing and compacting, as well as shredding used or obsolete components into pieces in order to recover some of the basic cell's building blocks. The construction of particular metabolites also generates a significant amount of leftovers that need to be dealt with. For example, 3′,5′-adenosine bisphosphate (pAp) is produced during sulphate assimilation and coenzyme A synthesis. In an attempt to characterise the proteins involved in its management, we purified an *E. coli* extract on a Sepharose-pAp column. This procedure retained several proteins. Among those we found both the CysQ protein, known to be a phosphatase, and the Orn protein, a nuclease degrading nanoRNAs (less than 5 nucleotides long) leftovers from RNA degradation (Mechold et al., 2007). Interestingly, both enzymes cope with leftovers of metabolism but in pathways previously not known to be related. Synthesis of coenzyme A as well as sulphate assimilation produce pAp. Control of gene expression requires RNA turnover, and nanoRNAs are produced continuously. They are degraded into the corresponding mononucleotides, that can be recycled into anabolic processes (RNA and DNA synthesis in particular) by nanoRNases.

Is this connection between quite different pathways random? What is the situation in other bacteria? Genome analysis uncovered counterparts of Orn in the proteome of gamma-Proteobacteria, but not in Firmicutes, Cyanobacteria or alpha-Proteobacteria. Yet degrading nanoRNAs is certainly an essential function (these very short RNAs fit the transcription and replication bubbles, and they will certainly interfere with transcription and replication). Uncovering a need creates a function, as we know in our daily experience. This certainly does not mean that the function is fulfilled by the same structure: in order to eat without soiling one's hand one may use a fork or a pair of chopsticks. We must understand that function (implying information) predates structure, although this is not a widely spread view. We therefore expected to uncover functional counterparts of Orn in all bacterial clades. And yes, complementation of an *E. coli orn* defect by libraries from a variety of bacterial genomes revealed proteins coming from unrelated structural descents, such as NrnA in Firmicutes. Remarkably, some were also able to hydrolyze pAp, i.e., could play the role that CysQ plays, substantiating the connection we had uncovered. Furthermore, organisms such as *Mycobacterium tuberculosis* have counterparts of both *orn* and *nrnA*, while they also have a *cysQ* gene showing that degradation of nanoRNA is very important in this organism with a complex lifestyle (very long persistent life in particular) (Postic et al., 2012). At the present time there are at least three different descents that lead to nanoRNAses, the Orn descent (also present in vertebrates), the NrnA descent (present in Firmicutes and Cyanobacteria) and the NrnC descent (present in alpha-Proteobacteria, Liu et al., 2012).

Paralogous metabolism

Metabolism is not a haphazard collection of chemical reactions. It is organised around a selected choice of building blocks and molecules acting as currencies for energy management. As all processes unfolding at 300 K metabolism is prone to make errors, creating a shadow metabolism that we named paralogous metabolism (Danchin and Sekowska, 2014) and that produces a variety of molecules which are dealt with via specific pathways. We illustrate this situation with a short list of paralogous compounds, and describe in details the specific situation of derivatives of a paralog of methionine, *S*-methyl-cysteine, which allowed us to characterise the way natural selection organised the corresponding chemical logic.

Paralogous compounds are recruited for new functions

As discussed above, proteinogenic L-amino acids are restricted to a short list of comparable compounds. It is obvious that chemically-related molecules must frequently interfere with the associated metabolic processes, and that errors keep happening at the level of translation (Perona and Gruic-Sovulj, 2014). Yet, the genetic code seems, at first sight, to be frozen. However, recruitment of new codons is certainly possible: this is how UGA was recruited to introduce in proteins selenocysteine, a paralog of cysteine, where selenium behaves as a paralog of sulphur (Brocker et al., 2014). This input favoured synthesis of selenomethionine, which can replace methionine with variable consequences, depending on the organism (Gojkovic et al., 2014). In the same way UAG was recruited to introduce pyrrolysine in proteins (Theil Have et al., 2013). Furthermore, niche isolation of specific organisms or even clades of organisms showed that the code keeps evolving. This can be seen with *Paramecium* protists (where UAG may code for glutamine, Preer et al., 1985) or *Candida* yeasts (where CUG may code for serine, Gomes et al., 2007). The main constraint that prevents the genetic code to evolve rapidly is likely to be the process of horizontal

gene transfer (HGT). In a context where cells behave as computers making computers (Turing Machines, Danchin, 2009), genes recruited by HGT would not be useful if they could not be accurately translated. By contrast, an advantage of codon ambiguity is alteration of protein surface antigens, allowing the organism to escape predators and/or escape the immune system of possible hosts. Also, there is further advantage for an organism to become genetically isolated as happens when the genetic code differs from the universal code, as this renders it immediately insensitive to a large number of viruses (which are genes deciphered using the standard genetic code). However this change comes at a cost that of losing the innovations brought about by horizontal gene transfer. As a consequence, the actual fixation of a particular genetic code is the result of the different trade-offs between all these constraints, and this results in a very slow evolution of the code, if any.

Besides L-selenocysteine as a paralog of L-cysteine, the emergence of D-amino acids as paralogs of their L-counterparts is the inevitable outcome of deamination processes that produce keto-acids from L-amino acids. These enantiomers are therefore commonplace and cells have enzymes proofreading the corresponding misacylation of tRNA (Wydau et al., 2009). However, in the Bacteria domain, cells recruited several of those as a way to make an envelope that would resist standard proteases (which have evolved to cope with L-amino acids) (Radkov and Moe, 2014). D-amino acids are also frequently recruited in the synthesis of antibiotics using non-ribosomal peptide synthesis (Hur et al., 2012).

A similar situation is encountered with D-carbohydrates. Inevitable racemisation has progressively opened up new functions that cope with accidental L-enantiomers. A case in point is the fate of L-rhamnose, which can be used in bacterial envelope (Aguirre-Ramirez et al., 2012), as are D-amino acids, thus escaping the most common carbohydrate degradation enzymes. However, in turn, this creates a novel niche for the organisms which evolved enzymes that can recognise these types of substrate (Rodionova et al., 2013), and the loop is opened to progressively evolve more complicated molecules, as well as novel enzymes.

Side reactions of standard pathways

We use here again the methionine salvage pathway as the illustration of an interesting paralogous reaction. In many organisms, including humans, a dioxygenase catalyses the last step before synthesis of KMTB, the immediate precursor of methionine (Fig. 4). This dioxygenase uses Fe^{2+} as a cofactor (Sparta et al., 2013). Surprisingly, the enzyme has two activities depending on its metal co-factor. When iron is involved, the enzyme produces KMTB, the immediate precursor of methionine, together with formate. In contrast, when nickel is involved, carbon monoxide is produced in addition to formate, together with 3-methylthiopropionate (Wray and Abeles, 1995). In *Bacillus subtilis* we found that CO was produced when the cells were grown in the presence of methylthioribose and nickel was added to the growth medium (Sekowska et al., 2004). Interestingly, this reaction exists in human cells, where CO is a gaseous mediator of inflammation and this should be explored when considering the harmful effects of the metal.

Even more interesting, a second enzyme of the methionine salvage pathway reveals paralogous reactions of considerable importance. The enolase MtnW that acts on 2,3-diketo-5-methylthiopentyl-1-phosphate is very similar ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the most abundant enzyme on earth (responsible for carbon fixation by plants), so much so that it can substitute for MtnW in relevant constructs, albeit with very poor efficiency (Ashida et al., 2005). Despite the fact that sulphur metabolism is very far from carbon fixation, it is remarkable that a paralogous reaction could have been recruited for one of the most important functions of living organisms on earth. This implies that it is essential to introduce enzyme promiscuity in reflections on the logic of metabolism.

The cysteine salvage pathway

The logic of metabolism reflects the history of living organisms. Amusingly, natural selection resulted in streamlining chemical processes in a way that is not very different from that brought about by human inventivity. We illustrate this fact in the protection/deprotection process involved in catabolism of *S*-methyl-cysteine (SMeC) in *B. subtilis* (Chan et al., 2014). Because this molecule contains a reactive sulphur atom, a straightforward prediction for SMeC catabolism was to assume that the oxygen-sensitive sulphur atom was the first site of reaction (Ohshiro et al., 2005). Oxidised sulphur would have been subsequently reduced by the sulphate/sulphite sulphur assimilation system. Another pathway could have used a beta-lyase activity generating methanethiol. However, experiments involving gene inactivation showed that *B. subtilis* did not produce a significant amount of methanethiol and that dioxygen was involved in the process (which would fit with the first hypothesis) but that the enzymes reducing sulphate were not involved (which rejects the hypothesis of sulphur oxidation). Furthermore, inactivation of a deformylase encoded by gene *defB* prevented the bacteria to use SMeC. An operon encoding monooxygenases was shown to be involved in the process. This led us to propose a scenario where the amino acid analogue is first protected by *N*-acetylation (preventing misincorporation into proteins), followed by an original step where the acyl-group is submitted to the action of monooxygenases. Subsequently, an *N*-deacetylation step results in an *S*-acylated product that transfers its acyl group to the amino group, as is the case of the OAS isomerisation to NAS (Lynch et al., 1994). Finally *N*-acyl cysteine is deacylated, yielding cysteine (Fig. 5).

This pathway is remarkable in that it proceeds exactly as does the chemist in her laboratory, protecting groups that need not be affected by subsequent steps and deprotecting the compound at the end of the process to produce the desired molecule.

Finale

While metabolism is at the core of the way life develops it is no longer fashionable, as many do not see the underlying logic that underscores the chemical transformations that

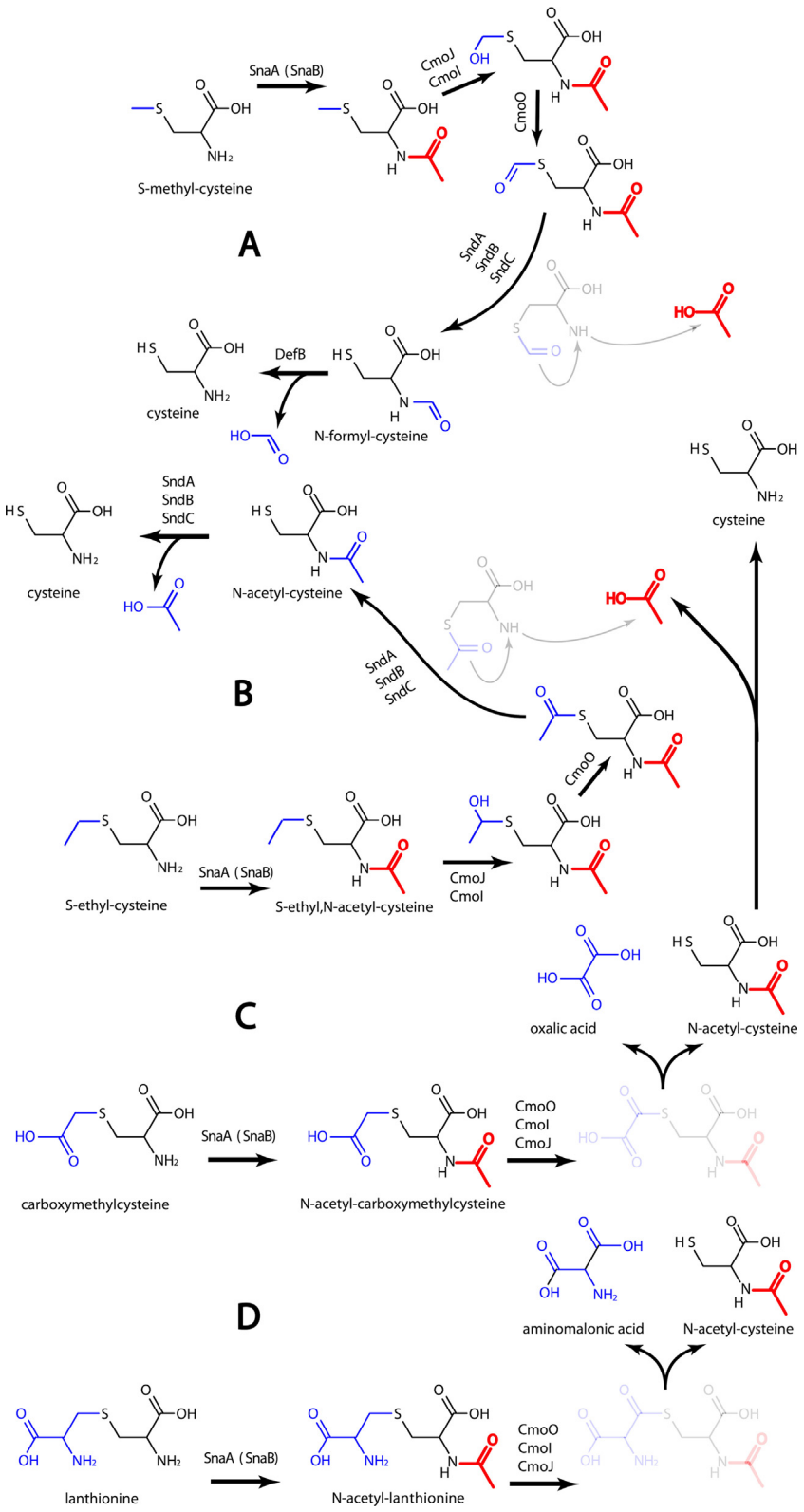

Figure 5 Catabolism of *S*-methyl-cysteine and related molecules. (A) Degradation of *S*-methyl-cysteine: the molecule is first ''protected'' by acetylation, then its methyl group is oxidised into a formyl group. The *S*-formyl acetylated molecule is ''deprotected'' by a deacetylase. Subsequently, the formyl-group migrates by cyclisation to the now free amino-group, and a deformylase liberates cysteine. (B) A similar process develops with *S*-ethyl-cysteine, where an acetyl-group plays a role similar to that of the formyl-group in (A), (C) and (D). Tentative scenario of the degradation of *S*-carboxymethyl-cysteine and lanthionine. In both cases it is likely that the oxidised intermediate is unstable and will be expelled out of the enzyme together with *N*-acetyl-cysteine. More work is necessary to explore the details of the reaction.

shape the cell's dynamics. Yet, while genes and enzymes vary from an organism (or even a tissue or a cell) to another one, metabolites stay the same. Looking at life processes will therefore benefit immensely from understanding how metabolic pathways are organised and how they are connected to one another. The short stories expounded in this article are but the tip of the iceberg, but we hope that they will entice the reader to find more.

Conflict of interest

The authors declare that there is no conflict of interest.

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